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Spatial analysis of the occurrence of protected butterflies in six European biogeographic regions as a tool for the environmental risk assessment of *Bt* maize

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Abstract

In the environmental risk assessment (ERA) of genetically modified plants (GMP), the consideration of the different environments where genetically modified plants (GMP) will be commercially grown (the receiving environments) plays a crucial role. In addition, relevant protection goals which may be adversely affected by the GMP have to be considered during the ERA. Using a literature- and GIS-based approach, distribution data of protected lepidopteran species listed in Council Directive 92/43/EEC and of maize cultivation was used in order to evaluate potential spatial overlaps between GM maize and protected non-target Lepidoptera in different biogeographical regions (BGR) of the EU. Each BGR has its peculiarity regarding maize cultivation and the distribution of protected butterflies. The lepidopteran fauna of the Pannonian BGR is particularly sensitive due to large maize cultivation shares and wide distribution of protected butterflies within this BGR. For the BGRs evaluated potential, spatial exposures of protected butterflies to GM maize cannot be excluded. This study shows that the suggested approach is a useful tool for the consideration of EU-wide protected species in different receiving environments during the problem formulation of the ERA of GMPs.

Keywords

Genetically modified plants, European Union, protection goals, environmental risk assessment, *Bt* maize, non-target Lepidoptera, biogeographical regions, Habitats Directive, spatial analysis

Introduction

In the European Union (EU), the authorisation of genetically modified plants (GMPs) is guided by principles which are laid down by Directive 2001/18/EC and its annexes. One of the basic principles in the authorisation system of GMPs in the EU is the case-by-case evaluation of the GMP in question. Potential adverse effects of the specific GMP on human health and the environment and the potential consequences thereof need to be identified and assessed during the environmental risk assessment (ERA). Detailed guidance on how to conduct the ERA of GMPs is provided by the European Food Safety Authority (EFSA 2010a, 2010b, 2010c, 2011a, 2016a, 2016b, 2016c).

An important aspect for the ERA of GMOs is that the receiving environments shall be taken into account when risk scenarios for the GMP in question are formulated and when data is generated by the applicant (EFSA 2010a). The receiving environments considered shall be representative of the environments where the GMP will be grown under commercial conditions (EFSA 2010a). The receiving environment is the environment into which the GMP will be released and the transgene may spread (EFSA 2010a). It is characterised by the GMP and its related management systems as well as the geographical zones with their different climates, soil conditions, flora and fauna (EFSA 2010a). For example, differences in the occurrence and distribution of non-target species in the different geographical zones shall be considered when selecting representative environments for ERA purposes (EFSA 2010c). Although there is no detailed guidance on how to define these receiving environments, the use of existing geographical zoning concepts has been proposed (EFSA 2010a, 2010c). Amongst others, the biogeographical regions of Europe have also been considered as a useful concept (EFSA 2010a). This biogeographical region concept was developed in order to define the biogeographical regions mentioned in Article 1 c) (iii) of the Council Directive 92/43/EEC (Habitats Directive, EC 1992), depicted in the Indicative Map of European Biogeographical Regions by the European Environment Agency (http://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe). This concept considers the most important factors which determine the distribution of epigeic organisms in Europe, e.g. vegetation and climate. Therefore it has been considered suitable for the classification of receiving environments when selecting test organisms for the ERA of GMPs (Jänsch et al. 2011). Practical experience with the use of biogeographical regions in the ERA of GMP is, however, largely limited. One study considered this biogeographical zoning concept for ERA purposes by identifying relevant earthworm species in arable soils in three European biogeographical regions (van Capelle et al. 2016). Two earthworm species were suggested as focal species for ERA testing as representatives of the Boreal and the Atlantic BGR. Another species was considered specifically relevant for the Mediterranean region (van Capelle et al. 2016). Another study selected the Continental biogeographical region when suggesting a selection procedure of focal species for ERA testing purposes (Hilbeck et al. 2014).

Another important aspect in the ERA of GMPs is that those aspects of the environment that need protection from harm need to be identified during the problem formulation step in the ERA (EFSA 2010a, 2010c). These so called environmental protection goals are set out by EU legislation and are also of relevance when selecting representative receiving environments for the ERA (EFSA 2010a, 2010c). EU-wide protection goals refer to biodiversity but also to specific environmental compartments such as soil, water and sustainable use of plant protection products or natural resources (EFSA 2010a, 2010c). Species of conservation concern have been recognised as relevant for the ERA of GMP due to their occurrence in agricultural landscapes (EFSA 2013, Schoonjans & Luttik 2014). In particular, the conservation of species and habitats listed in the Annexes of Directive 92/43/EEC (EC 1992) is one of the priorities to halt the loss of biodiversity in Europe (EC 2006, EC 2011). Consequently, discussions have focused on how to consider endangered and protected non-target species in the ERA of GMP. Suggestions for the selection of these species for ERA testing purposes were made by Hilbeck et al. (2014). Other authors suggested using ERA testing results from species that are biologically or taxonomically close instead of testing protected or endangered species (Dolezel et al. 2011). EFSA also suggested the use of taxonomically related surrogate species for covering endangered species in the ERA (EFSA 2013). EFSA's Scientific Committee recently dedicated a Scientific Opinion to the coverage of endangered non-target species in the environmental risk assessment (EFSA 2016c).

A prominent example of non-target species of conservation concern are non-target butterflies which are recognised as important protection goals when assessing environmental risks of insect resistant (*Bacillus thuringiensis*, *Bt*) maize. In maize fields, few butterfly species occur and these are mostly considered as pest species (Gathmann et al. 2006). Therefore all assessments of effects of *Bt* maize on non-target butterflies refer to butterfly populations occurring in off-field habitats such as field margins or natural habitats interspersed with cropland. In arable regions where maize is cultivated, different habitat types are interlinked with intensively managed arable fields (Heissenberger et al. 2003). In case studies in Austria, Germany and Switzerland, butterflies of conservation concern were observed in agro-environments where maize is cultivated (Aviron et al. 2009, Lang 2004, Traxler et al. 2005). In addition, small-scale nature reserves and protected habitats which provide important habitats for species protected under the Habitats Directive (FFH species) can be interspersed in agricultural landscapes where maize is cultivated (Lang et al. 2015). These semi-natural and natural habitats are important refuges for already depleted butterfly communities in agro-environments (Thomas 2016, Traxler et al. 2005). Maize pollen expressing *Cry* proteins deposited on to host plants of butterfly larvae near maize fields can adversely affect butterfly larvae when feeding on host plant leaves by inadvertently ingesting transgenic pollen (Dively et al. 2004, Felke et al. 2002, Hellmich et al. 2001, Lang and Otto 2010, Lang and Vojtech 2006). Consequently, non-target Lepidoptera are routinely addressed in the ERA of *Bt* maize in Europe (EFSA 2011b, 2011c, EFSA 2012a, 2012b).

However, endangered or protected butterfly species are often difficult to detect under field conditions due to the low abundances of certain species which require higher sampling efforts than frequent species (Lang 2004, Lang and Bühler 2012, Lang et al. 2016). In addition, laboratory testing of these species is either difficult or undesirable (EFSA 2016c). Current ERA practice is to use modelling approaches for evaluating risks to non-target butterflies, considering a wide range of sensitivities of butterflies to the *Cry*-toxin (EFSA 2012a, 2012b, Perry et al. 2010, Perry et al. 2012). Risk management options have been proposed to address adverse effects of *Bt* maize on Lepidoptera of conservation concern specifically occurring in protected habitats in agro-environments (e.g. EFSA 2012a, 2012b, 2015). However, scientific controversies remain on the isolation distances and buffer zones necessary to protect these species (Hofmann et al. 2016, Lang et al. 2015).

How to address different receiving environments in the ERA of GMP was one of the aims of the research being undertaken in the EU-project AMIGA (Assessing and Monitoring the Impacts of Genetically modified plants on Agro-ecosystems). A major task in the context of AMIGA was the improvement of baseline data on biodiversity in selected agro-ecosystems and adjacent habitats in five different geographical regions of Europe (Arpaia et al. 2014). This included, amongst others, the identification of region-specific characteristics, including regional protection goals, in order to support the selection of receiving environments for the ERA of GMP (Arpaia et al. 2014).

This study presents research carried out in the course of the AMIGA project. The aim was to identify and assess relevant protection goals in different receiving environments of Europe for the ERA of GMPs. Butterfly species protected under the Habitats Directive were used as a case study representing EU-wide protection goals for biodiversity as well as important non-target species when cultivating insect-resistant *Bt* maize in the European Union. The protected butterfly species are often restricted in their range, have a very local occurrence and may therefore not be affected by the GMP due to geographical distributions other than the cultivation area of the GMP in question. Assessing the spatial overlap between a particular GMP and protected or endangered species during the problem formulation of the ERA would allow identification of those species of conservation concern that are likely to be exposed to *Bt* maize and those without potential occurrences in or next to GMP cultivation areas. For this purpose, European butterfly species protected by the Habitats Directive were evaluated whether they may occur within maize cultivation areas of Europe using the biogeographical region approach. A similar approach could also be used for other protected species (e.g. protected moth species) or even habitats in close spatial relationship to the cultivation of GMPs. Habitats protected by the Natura 2000 system under the Habitats Directive represent protection objects with specific conservation goals which may also be affected by the cultivation of GMPs. By using a similar methodology, the spatial relationship between Natura 2000 habitats and potential GMP cultivation could also be studied. For the demonstration of the approach shown in this study, the authors focus on protected butterfly species under the Habitats Directive.

The specific questions addressed in this study were:

Are the European biogeographical regions a useful concept for classifying receiving environments for the ERA of GMPs?
Can potential spatial overlaps between protected FFH butterfly species and maize cultivation areas in different biogeographical regions be identified by the use of a GIS-based spatial analysis?

Methods

Maize cultivation data

From the EUROSTAT online database, the areas of maize cultivation (green maize and corn maize) were queried for NUTS1 and NUTS2 regions, using the latest available data entry between 2003 and 2012 for each country or region (http://epp.eurostat.ec.europa.eu). The NUTS classification (Nomenclature of Territorial Units for Statistics) is a hierarchical system for dividing up the economic territory of the EU for the purpose of harmonised statistics and regional socio-economic analyses. NUTS1 is the level of major socio-economic regions. The next smaller units of NUTS level 2 correspond to *Regioni* (IT), *Régions* (FR), *Regierungsbezirke* (DE) or *Bundesländer* (AT) (http://ec.europa.eu/eurostat/web/nuts/overview). If available, the maize area of NUTS2 was used, otherwise the proportional split of the NUTS1 maize area was used for those regions not covered by NUTS2 data. Maize data from Greece and Cyprus was not available. Maize data was used from EU Member States for which data on the distribution of protected butterflies was available (see also below). In case the border of the biogeographic region separated a NUTS2 region, the maize cultivation area of the NUTS2 region was split between the two neighbouring biogeographic regions in proportion to the biogeographic region area share assuming equal distribution of maize in the NUTS2 region. Results are presented as hectares maize cultivation per total area of the biogeographic region or NUTS2, respectively.

Selection of butterfly species

All butterfly species listed in Annexes II and IV of the Habitats Directive were used as a starting point for the analysis. Of the 31 butterfly species listed in the Annexes, 29 species were selected and two species were excluded (*Polyommatus eroides* and *Hesperia comma* f. *catena*). *Polyommatus eroides* is considered a subspecies of *P. eros*. *Hesperia comma catena* is considered a subform of *H. comma*. Both species are not listed in the Annexes of the Habitats Directive (van Swaay et al. 2012).

Species occurring exclusively at high altitudes (above the timberline) or in high geographical latitudes are less likely to spatially overlap with maize cultivation areas and were therefore also excluded. This was the case for species which occur near or above

the timberline: *Clossiana* (*Boloria*) *improba*, *Erebia sudetica*; at high altitudes: *Erebia christi*, *Erebia calcaria*; or in Arctic regions: *Erebia polaris*, *Agriades* (*Plebejus*) *glandon aquilo*. Also *Plebicula* (*Polyommatus*) *golgus* was excluded as this species only occurs in two mountain chains in southern Spain at high altitudes. For the species *Nymphalis vaualbum* and *Pseudophilotes* bavius, no Article 17 assessments were available for the first reporting period (http://bd.eionet.europa.eu/activities/Reporting/Article_17). These nine species were therefore not considered in the analysis.

Distribution data of butterfly species protected under the Habitats Directive

Data on the distribution of the selected 20 FFH butterfly species was retrieved from the Article 17 Report Database of the EEA (www.eea.europa.eu/data-and-maps/data/article-17-database-habitats-directive-92-43-eec). National distribution data was transposed to a 10 km grid due to the heterogeneity of the data sets received. At the time of the compilation of this manuscript, only data from the first reporting period (2001 – 2007) under article 17 were available. No information on the distribution of FFH butterfly species from five EU Member states (Romania, Bulgaria, Croatia, Malta and Cyprus) was available. Therefore FFH butterfly data cover EU 23.

The distribution data of the 20 FFH butterfly species was used to calculate the share of each BGR area occupied by a specific FFH species occurring in this BGR. Boxplots indicating the median, the quartiles and the interquartile range were plotted. The share of the distribution area of each FFH butterfly species in each BGR was calculated in order to evaluate the importance of each BGR for the individual species.

Biogeographical regions (BGRs)

The zoning scheme of the BGRs of Europe comprises 9 biogeographic regions according to Article 1 of Directive 92/43/EEC (http://www.eea.europa.eu/data-and-maps/data/biogeographical-regions-europe; version of 25. Apr 2011). As the BGRs cover the geographical Europe, data have been clipped to the area of the available maize and FFH butterfly distribution data. The Macaronesian, Black Sea and the Steppic BGRs are not represented in this evaluation due to a lack of data on FFH butterfly distribution from these regions in the first Article 17 reports. In total, 6 BGRs were taken into consideration representing the study area (Figure 1).

Spatial overlap between FFH butterfly species and maize cultivation

The spatial overlap between the distribution areas of FFH butterfly species and the maize cultivation in a particular BGR was shown by the use of two case studies. The distribution areas of the FFH butterfly species *Euphydryas aurinia* and of *Lycaena helle* in a specific BGR (Atlantic BGR and Boreal BGR, respectively) were plotted against

Figure 1. The six biogeographic regions of Europe in the study area. Source: European Environment Agency.

data on the maize cultivation in the respective BGR in order to assess the spatial overlap and the potential for exposure to *Bt* maize cultivation in the specific BGR.

All geographic data were processed using the GIS software package ArcGIS/ArcMap, version 10.2.

Results

Maize cultivation in the biogeographic regions of Europe

The area of maize cultivation is not equally distributed across the different biogeographical regions of Europe (Table 1). The total maize area in the six BGRs amounts to more than 10 Mio hectares, representing approximately 2.6% of the total BGRs area.

Although the majority of the maize cultivation area is located in the Continental BGR, with more than 3 Mio hectares maize, the Pannonian BGR has the highest share of maize. The Boreal BGR has the smallest maize area and share of maize.

If the maize share is broken down on the level of the NUTS2 regions, the core areas of maize cultivation in Europe can be seen (Figure 2). Maize cultivation is concentrated in the Pannonian biogeographic region. This region has the highest median, first and third quartile of the NUTS2 region maize shares (Figure 3). The large interquartile range in the Pannonian BGR indicates that the maize cultivation share in this biogeographical region is considerably varying across the NUTS2 regions. In addition, the cumulative maize area in relation to the total biogeographical area shows that the maize cultivation in the Boreal, Atlantic and in the Alpine BGR is concentrated in few NUTS2 regions (Figure 4). Particularly in the Boreal BGR, 90% of the maize cultivation area is concentrated in approximately 20% of the BGR`s area. In contrast, in the Continental, Pannonian and Mediterranean BGRs, maize cultivation is more widely distributed across the NUTS2 regions.

Distribution of FFH butterflies in the biogeographic regions of Europe

After exclusion of 11 FFH butterfly species (see Methods) the remaining 20 butterfly species were spatialised to six biogeographic regions of Europe according to their reported distribution (Table 2). The alpine BGR hosts 17 FFH lepidopteran species. Ten species occur in the Atlantic and Boreal region respectively. The continental BGR hosts 17 species, the Mediterranean 14 species. In the Pannonian region, 13 species are present. Five species occur across all biogeographic regions (*M. teleius*, *M. arion*, *E. aurinia*, *L. achine*, *L. dispar*) while three species are endemic to the Mediterranean BGR (*F. elisa*, *M. arge*, *P. hospiton*).

According to the Habitats Directive, the conservation of Article II species requires the designation of Special Areas of Conservation while Annex IV species require strict protection across their natural range. From the Article 17 report, database information is also available to what extent the populations of the individual FFH butterfly species listed in Annex II are covered by Natura 2000 sites in each EU Member State. For 9 out of the 20 butterflies addressed in this study information is available on the coverage of their populations by Natura 2000 sites (Table 2).

The calculated shares of the BGR area occupied by the different butterfly species in the different BGRs are shown in Figure 5. Three groups can be distinguished. In the first group of BGRs (Atlantic and Mediterranean BGR), the FFH butterfly species have the smallest distribution areas as shown by the smallest median and interquartile ranges of the distribution area shares. The largest distribution area share in both BGRs is represented by only one species (*E. aurinia*, 13.1% and 9.6% respectively, of each BGR). The second group (Boreal and the Continental BGR) has an intermediate interquartile range (4.9% and 6.4%, respectively) of the BGR area

Table 1. Area, maize cultivation area and calculated maize share in six biogeographic regions of Europe.

BGR	Area (ha)	Maize (ha)	Maize share (%)
Alpine	30,302,147	875,338	2.9
Atlantic	78,180,467	3,369,596	4.3
Boreal	83,915,636	43,326	0.1
Continental	103,595,103	3,709,289	3.6
Mediterranean	74,501,738	697,812	0.9
Pannonian	11,074,931	1,374,209	12.4
Total	381,570,022	10,069,570	2.6

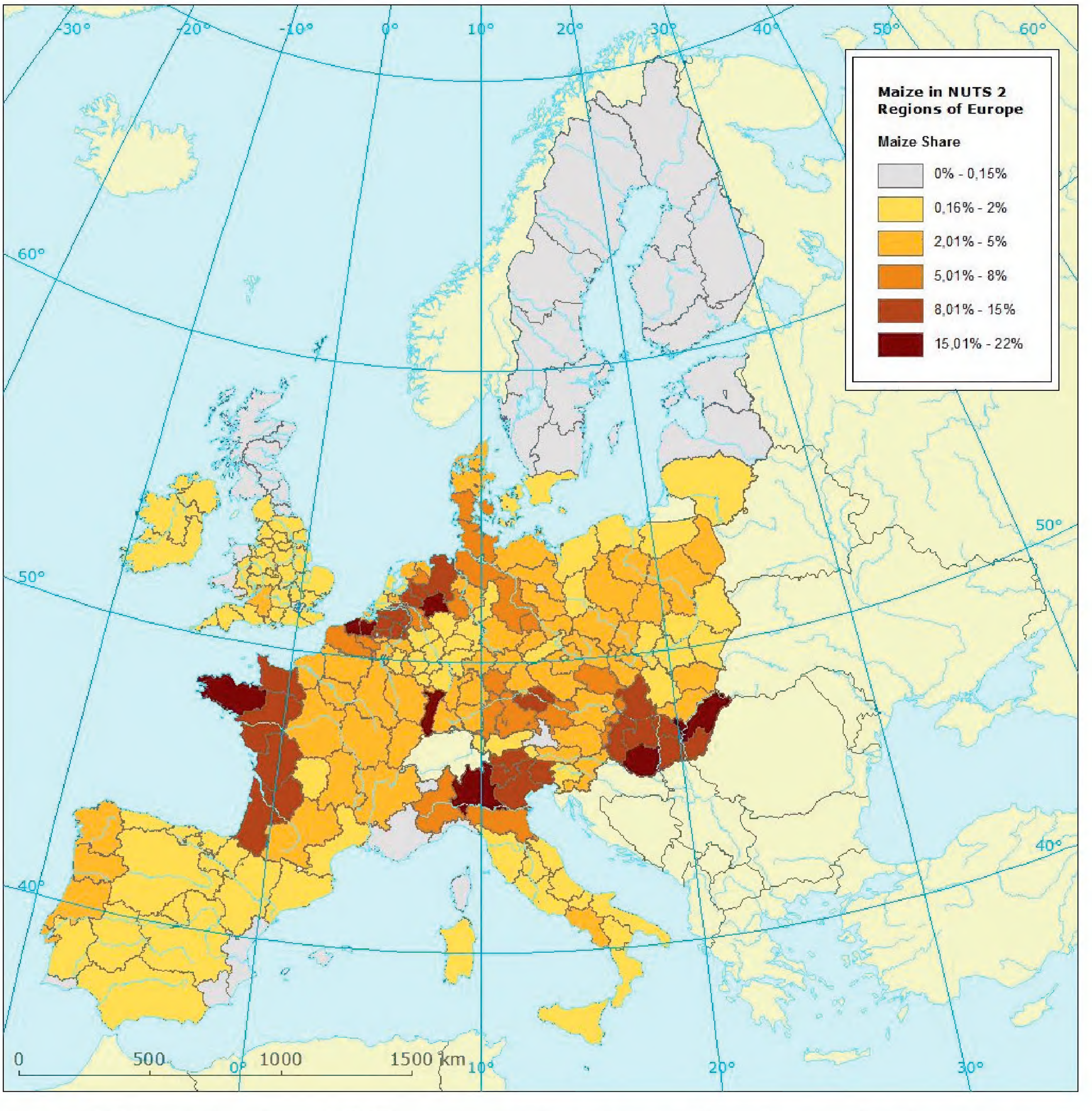

Figure 2. Maize cultivation in NUTS2 regions of the study area (share of maize of total area in %).

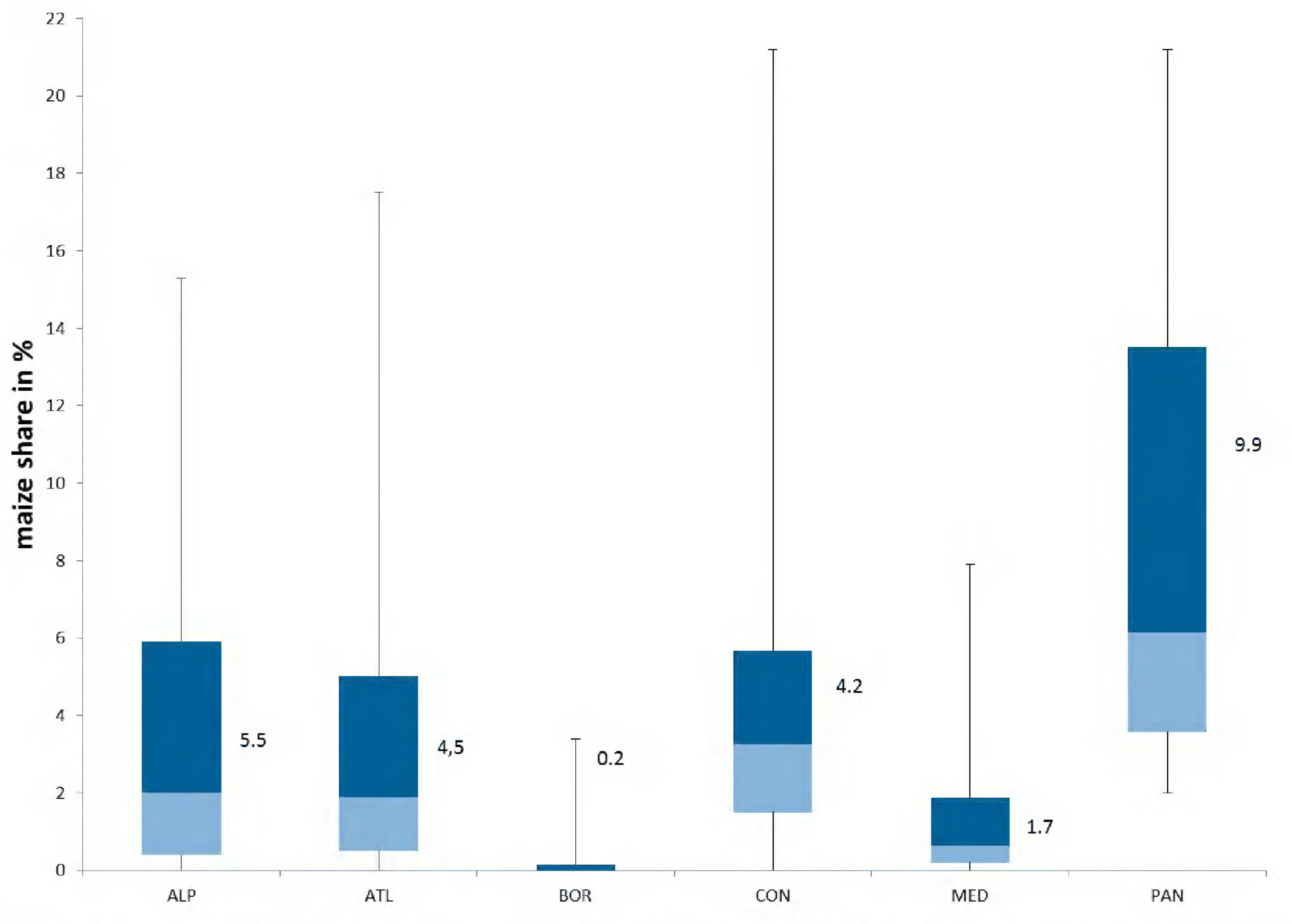

Figure 3. Boxplots of maize shares in NUTS2 regions of six different BGRs in Europe. Light blue box indicates lower (25%) quartile to median value. Dark blue box indicates median values to upper (75%) quartile. Whiskers indicate minimum and maximum values. Numbers next to boxes indicate interquartile range of maize shares of NUTS2 regions in each BGR. All values refer to percentage of maize of NUTS2 regions in the specific BGR.

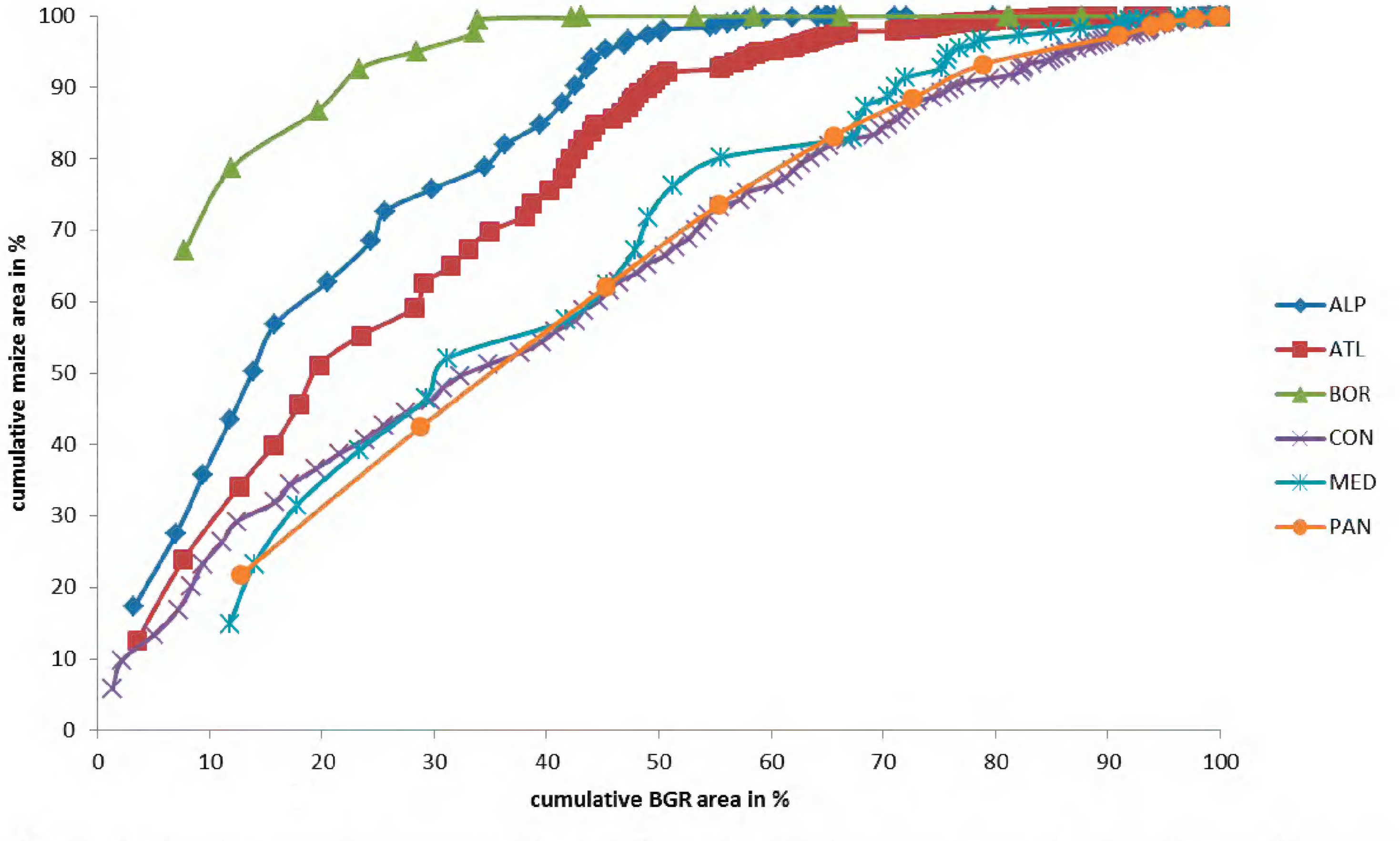

Figure 4. Cumulative maize area in relation to the biogeographical area in six biogeographic regions of Europe.

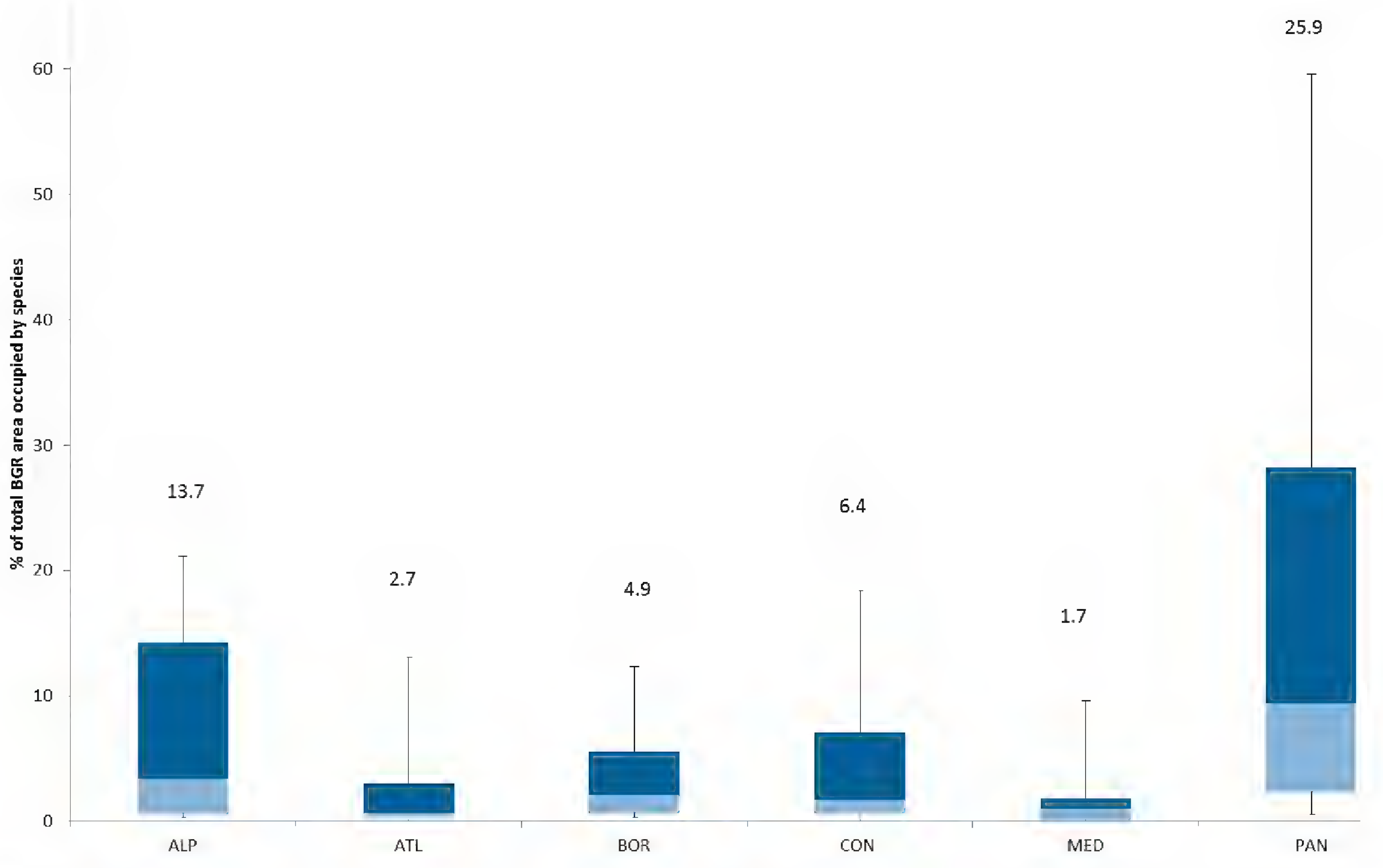

Figure 5. Share of BGR area occupied by lepidopteran FFH species occurring in this BGR in the 6 BGRs. Light blue box indicates lower (25%) quartile to median value. Dark blue box indicates median values to upper (75%) quartile. Whiskers indicate minimum and maximum values. Numbers above whiskers indicate the interquartile range. All values refer to percentage of the BGR area occupied by the species.

shares of their FFH butterfly species and a similar median (2.1% and 1.7% respectively). However, FFH butterfly species in the Continental BGR have a slightly larger variability in their BGR area shares. The third group, comprising the Alpine and Pannonian BGR, is characterised by larger area shares compared to all other BGRs. Particularly, in the Pannonian BGR the shares of the BGR area occupied by FFH butterflies are larger than in all other BGRs. This BGR hosts most species with large shares of the BGR area.

Calculating the share of the total distribution area of each FFH butterfly species in each of the BGRs, the importance of each BGR for the distribution of the respective species can be seen (Table 3). The distribution areas of *F. elisa*, *P. hospiton* and *M. arge* are concentrated in the Mediterranean BGR. *Fabriciana elisa* and *P. hospiton* are endemic to Corsica and Sardinia, respectively. *Melanargia arge* is endemic to central and southern Italy which encompasses the Mediterranean and the Alpine BGR. *Apatura metis* occurs almost exclusively in the Pannonian BGR. Most other species have their distribution scattered over two or more BGRs. The continental BGR has the highest number of species with large distribution area shares. In contrast, the Pannonian and the Atlantic BGRs host the fewest species with considerable shares of distribution in these BGRs (Table 3).

Table 2. Occurrence of 20 FFH butterflies in six biogeographic regions of Europe. Distribution data from EEA. x = occurrence; **ALP** = Alpine; **ATL** = Atlantic; **BOR** = Boreal; **CON** = Continental; **MED** = Mediterranean; **PAN** = Pannonian; **Annex** = listed in Annex II or IV of Directive 92/43/EEC; **Natura 2000** = Information on the proportion of the population covered by the Natura 2000 network; x = reported (x) = not reported.

Species	Annex	ALP	ATL	BOR	CON	MED	PAN	Natura 2000
Apatura metis	IV				x	x	x	
Coenonympha hero	IV	x	x	x	x			
Coenonympha oedippus	II, IV	x	x		x		x	x
Colias myrmidone	II, IV	x			x		x	x
Euphydryas aurinia	II	x	x	x	x	x	x	x
Fabriciana (Agrynnis) elisa	IV					x		
Hypodryas (Euphydryas) maturna	II, IV	x	x	x	x		x	x
Leptidea morsei	II, IV	x			x		x	x
Lycaena dispar	II, VI	x	x	x	x	x	x	x
Lycaena helle	II, IV	x		x	x			x
Lopinga achine	IV	x	x	x	x	x	x	
Maculinea (Phengaris) teleius	II, IV	x	x	x	x	x	x	x
Maculinea (Phengaris)) arion	IV	x	x	x	x	x	x	
Maculinea (Phengaris) nausithous	II, IV	x	x		x	x	x	x
Melanargia arge	II, IV	x			x	x		(x)
Papilio alexanor	IV	x				x		
Papilio hospiton	II, IV					x		(x)
Parnassius apollo	IV	x	x	x	x	x		
Parnassius mnemosyne	IV	x		x	x	x	x	
Zerynthia polyxena	IV	x			x	x	x	

Table 3. Share of distribution area of FFH butterflies in the respective BGR (percentages).

	ALP	ATL	BOR	CON	MED	PAN	Total
Apatura metis	0.1	0.0	0.0	0.0	0.0	99.9	100
Coenonympha hero	3.6	0.0	65.1	31.3	0.0	0.0	100
Coenonympha oedippus	19.0	32.6	0.0	45.2	0.0	3.1	100
Colias myrmidone	12.9	0.0	0.0	80.7	0.0	6.4	100
Euphydryas aurinia	17.1	28.9	4.6	27.7	20.3	1.4	100
Fabriciana elisa	0.0	0.0	0.0	0.0	100.0	0.0	100
Hypodryas maturna	6.3	0.0	64.2	10.2	0.0	19.3	100
Leptidea morsei	35.2	0.0	0.0	41.1	0.0	23.8	100
Lopinga achine	35.0	0.4	27.1	31.5	0.1	5.8	100
Lycaena dispar	8.6	7.3	23.2	44.4	0.2	16.4	100
Lycaena helle	3.7	0.0	18.6	77.6	0.0	0.0	100
Melanargia arge	6.5	0.0	0.0	1.8	91.8	0.0	100
Maculinea arion	33.8	1.6	8.8	42.0	7.0	6.8	100
M. nausithous	7.7	1.8	0.0	84.5	0.2	5.8	100
M. teleius	11.7	3.3	3.6	62.0	0.7	18.8	100
Papilio alexanor	43.0	0.0	0.0	0.1	57.0	0.0	100
Papilio hospiton	0.0	0.0	0.0	0.0	100.0	0.0	100
Parnassius apollo	65.6	5.1	3.4	10.7	15.1	0.0	100
Parnassius mnemosyne	38.1	0.0	18.4	19.0	4.5	20.0	100
Zerynthia polyxena	11.4	0.0	0.0	34.3	18.5	35.8	100

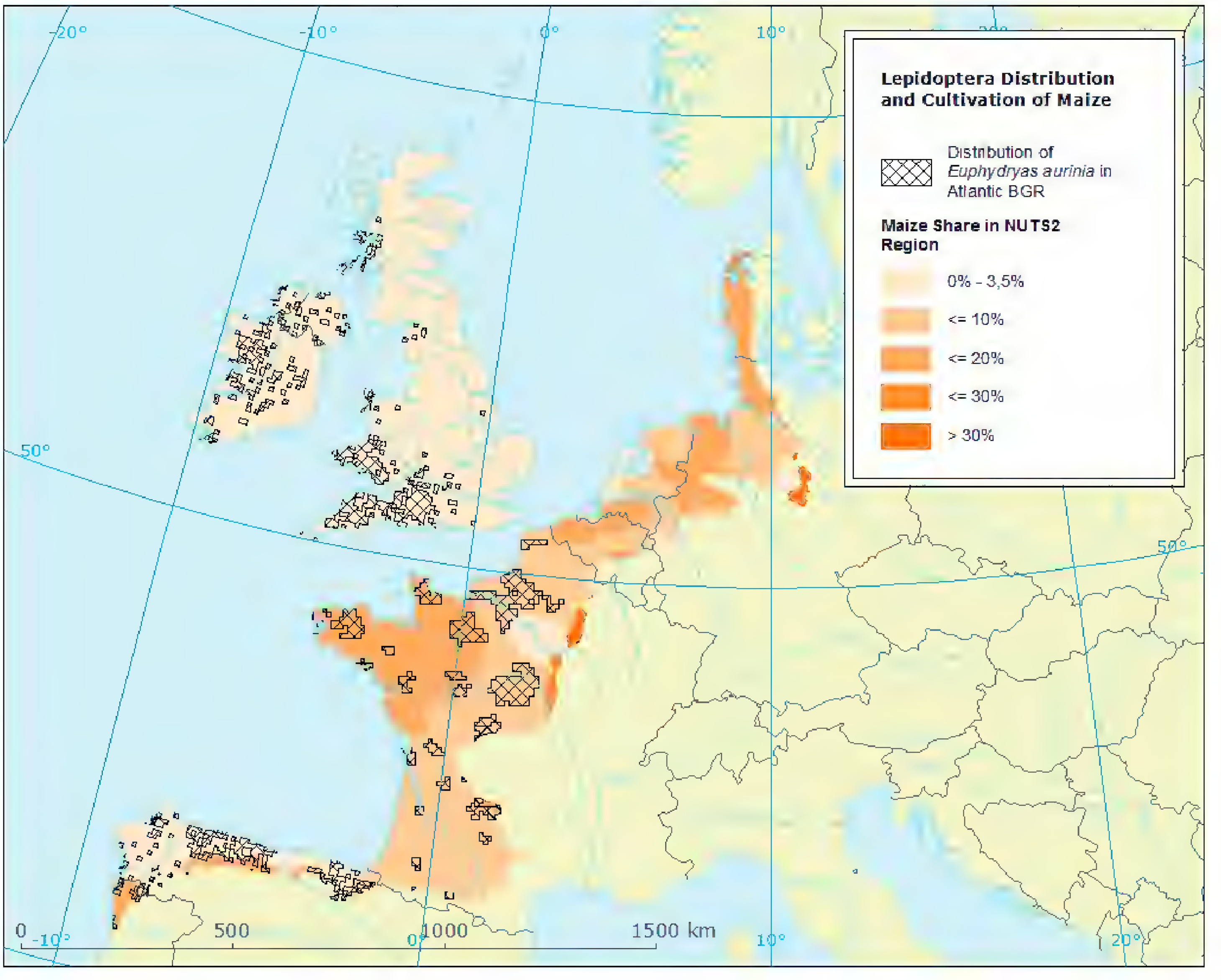

Figure 6. Distribution of the lepidopteran FFH species *Euphydryas aurinia* and share of maize in the Atlantic BGR.

Examples of spatial overlap of maize cultivation areas with the distribution area of FFH Lepidoptera

The spatial overlap between the distribution areas of two FFH butterfly species and maize cultivation in a specific BGR are shown in Figure 6 and Figure 7. In the Atlantic BGR, the distribution of *Euphydryas aurinia* is restricted to certain EU Member States (e.g. Spain, France, UK) while it does not occur in Belgium, the Netherlands, Germany and Denmark. Maize is cultivated throughout the Atlantic BGR. However, the majority of the distribution of *Euphydryas aurinia* in the Atlantic BGR lies in areas with low maize cultivation (United Kingdom, north of Spain). Distribution areas of this species can also be identified within NUTS2 regions in France with higher maize shares. This indicates that areas of potential spatial proximity between the distribution of the species and maize cultivation exist for this species in this BGR.

As a second case study, the distribution of *Lycaena helle* in the Boreal BGR is shown (Figure 7). The majority of the distribution area of this FFH butterfly species is located in Sweden and Finland where maize cultivation is virtually absent. Maize cultivation in the boreal distribution area of *L. helle* is restricted to one NUTS2 region (Lithuania).

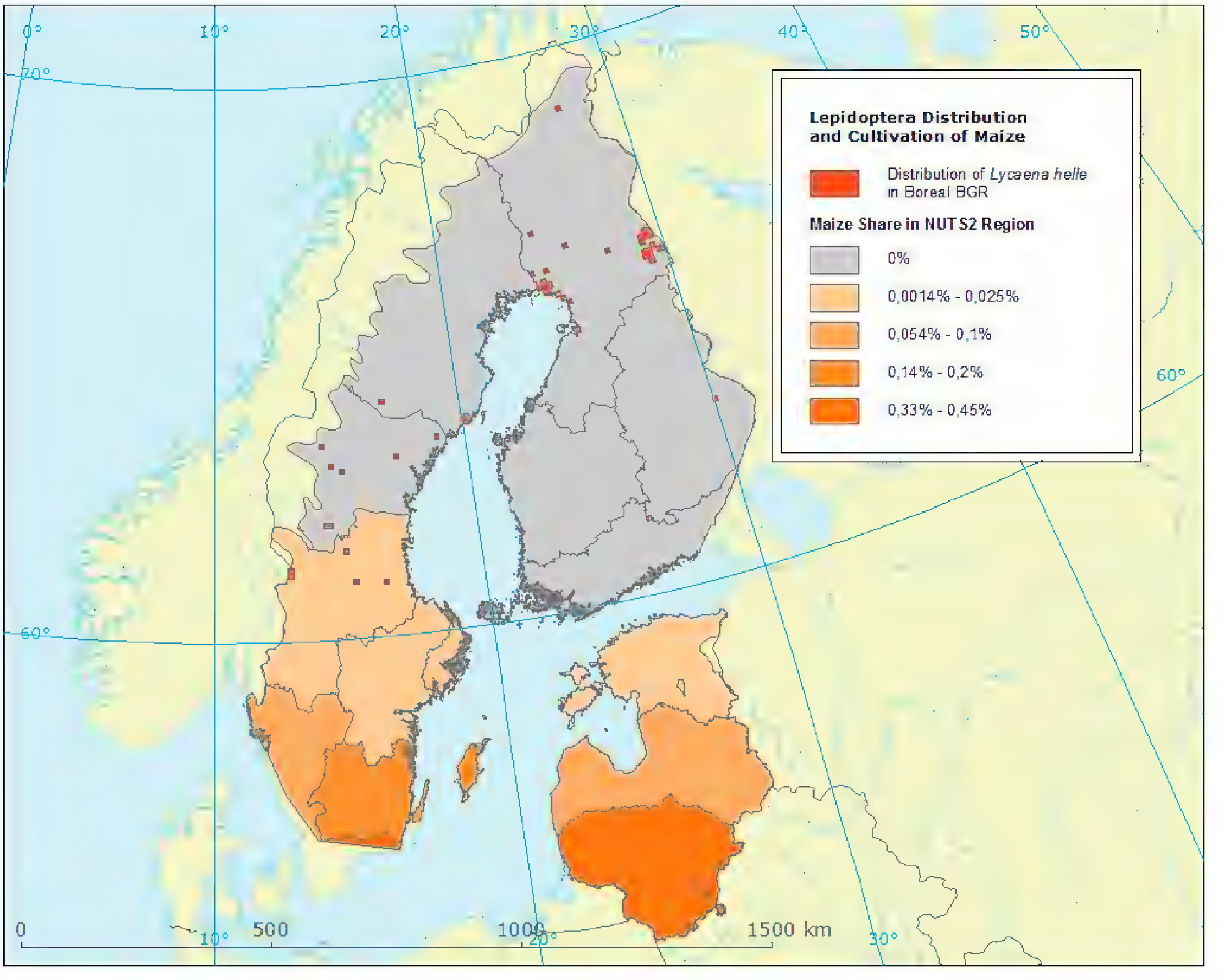

Figure 7. Distribution of the lepidopteran FFH species *Lycaena helle* and share of maize in the Boreal BGR.

Discussion

Are the European biogeographical regions a useful concept for classifying receiving environments for the ERA of GMPs?

The ERA of GMPs has to be carried out on a case-by-case basis, meaning that the required information varies depending on the types of the GM plants and trait(s) concerned, their intended use(s) and the potential receiving environment(s) where the GMP is intended to be grown (EFSA 2010a). The classification of receiving environments during the ERA of GMPs is needed in order to consider different agronomic and environmental conditions across the EU in the ERA which GM plant may encounter if cultivated in the EU. These specific agro-environmental conditions have been thoroughly characterised for the biogeographic regions (see EEA 2002). These described differences in agro-environmental conditions across the EU imply that potential differences in exposure and impacts of a particular GMP, depending on the specific environment, can be expected. Therefore the applicant of a GMP is required to select testing sites where exposure and impacts are expected to be highest (EFSA 2010a). The use of the Indicative Map of European Biogeographical Regions

as a zoning concept for the ERA of GMP has the advantage that datasets of the species` distributions in the different biogeographical regions are made available by the European Topic Centre on Biological Diversity (ETC/BD 2013). If these data are combined with data on the cultivation of the relevant genetically modified crops in the different BGRs, the potential spatial overlap of FFH species and crop cultivation in the different biogeographic regions of Europe can be quickly evaluated. This information can be used in the problem formulation of the ERA in order to select those BGRs where the potential exposure of the respective non-target organisms to the GMP and therefore the potential impact may be highest. However, it has to be emphasised that such an evaluation does not cover all protected FFH lepidopteran species, due to limitations in the availability and entirety of the species distribution data at the EU level. In addition, some Member States have not fully met their reporting tasks and, during harmonisation of the data in order to generate an EU dataset, some information details have been omitted. In addition, as the reporting requirements of Article 17 refer only to the presence or absence of species in the respective spatial grid, there is no information on the population size or density of the species in the datasets.

However, the repeated reporting cycle in the framework of Article 17 of the Habitats Directive has the potential that the reported data quality and entirety will be improved during the next reporting periods, so that a repeated analysis is likely to produce more reliable results. Even if data for specific species in particular BGRs are incomplete or lacking, the approach can be useful for a first assessment of potential spatial overlaps during the problem formulation of the ERA, although it must be kept in mind that the results should be interpreted cautiously.

Can potential spatial overlaps between protected FFH butterfly species and maize cultivation areas in different biogeographical regions be identified by the use of a GIS-based spatial analysis?

The analysis based on the BGR classifications shows that each BGR has its specificities regarding the occurrence and distribution of FFH butterfly species and maize cultivation. The Continental BGRs is the largest of the European BGRs which may explain why it hosts the highest number of species with a significant share of their distribution in this BGR. However, the Mediterranean BGR, although generally having low shares of distribution areas of butterflies, is of importance for certain species as their distribution areas lay exclusively within this BGR. Distribution ranges of FFH butterfly species occurring in the Boreal BGR, the second largest region, as well as maize cultivation, are minimal in this BGR. The analysis excluded species occurring above the timberline, thus the FFH butterfly species relevant for the Alpine BGR are likely to be concentrated in the inner-alpine valleys or alpine foothills where maize, in particular silage maize for cattle feeding, can still be grown (Statistik Austria 2015). The Pannonian BGR plays an important role for FFH butterfly species as evidenced by the fact that many of the species are widely distributed within this BGR. The Pannon-

ian BGR has the highest maize share of all BGRs, possibly due to the favourable climatic conditions and the availability of humus-rich soils (EEA 2002). This BGR also has the highest proportion (67%) of regularly cultivated habitats compared with any other of the BGRs (EEA 2002). Maize cultivation is therefore more evenly distributed across this BGR compared to other BGRs in which maize cultivation is concentrated within a few NUTS2 regions.

Maize is cultivated in all six BGRs assessed in this study, although with a varying intensity between and, particularly, within the BGRs. While maize cultivation is limited in the Boreal and the Mediterranean BGR, the Pannonian BGR is a maize cultivation hotspot within the EU. Limiting factors for maize cultivation are low soil temperatures for germination, low temperature sums and a lack of sufficient precipitation in the summer months (AGES 2015), factors which also limit its cultivation at higher geographic latitudes (Odgaard et al. 2011). Therefore only FFH butterfly species which are restricted in their occurrence to high geographical latitudes or high altitudes where maize cultivation is absent, may have a low likelihood to come into contact with maize cultivation and therefore *Bt* maize pollen in case *Bt* maize is cultivated. However, dynamics in the distribution of maize areas may change with changes in climatic factors (Odgaard et al. 2011). Major agro-climatic changes predicted due to climate change will affect yield-limiting factors in the crop production in Europe (Eitzinger et al. 2012), thereby possibly changing regional crop production patterns. For example, maize yields in the lowlands of Slovakia are predicted to decline under different climate scenarios until the year 2100 due to the fact that maize is more affected by drought and will experience less fertilising effect of increasing CO_2 concentrations (Eitzinger et al. 2012). However, also agronomic factors such as the occurrence of important pest species and the development of new maize varieties (e.g. silage maize) may also extend maize cultivation to climatic areas where currently no maize can be grown (Hein 2002). It has also to be kept in mind that maize cultivation is also significant in other BGRs not covered by this study, in particular the Steppic region and may be relevant for future assessments of risks for protected butterfly species in European agro-ecosystems.

When using the European biogeographic regions concept, potential spatial overlaps for FFH butterfly species can only be excluded if either the particular FFH butterfly species or maize cultivation is absent from the specific biogeographic region. At this geographical scale, results could be different for other crops such as sugar beet which is highly concentrated in north-western Europe (CIBE/CEFS 2003). Increasing the spatial resolution for maize cultivation data as well as the species` distributions to the NUTS2 level allows more detailed conclusions about potential spatial coincidences between FFH butterfly species and maize cultivation. In practice, however, spatially explicit information and analyses, at even finer geographical scales than the NUTS2 regions, would have to complement the assessments at the BGR and NUTS2 level in order to derive robust conclusions for the respective FFH butterfly species.

The methodology proposed in this study allows a first assessment of potential spatial overlaps of FFH butterflies and maize cultivation during an EU-wide risk assessment approach. However, uncertainties remain with respect to the factual exposure of

the individual species to *Bt* maize. Risks for individual butterfly species due to *Bt* maize cultivation depend not only on spatial exposure of the butterfly to *Bt* maize pollen but also on the individual biological characteristics and regional life cycle specificities of the particular butterfly species. For example larvae of certain species (e.g. *Maculinea* spp.) are social parasites of different ant species (Elmes et al. 1998, van Swaay et al. 2012) and spend much of their lifetime in the ants` nests; thereby larvae are most likely not exposed to maize pollen even if maize is grown adjacently. For other species the temporal coincidence of larval stages with maize pollen shed is rather unlikely. For example, first instar larvae of *Parnassius apollo* occur in early spring (Bauer and Feurle 2017) and pupate most likely before maize pollen is shed. As this species is univoltine, the larvae are less likely to come into contact with *Bt* maize pollen. Hence, the specific phenology of the larvae in a particular biogeographic region as well as the number of generations per year are important factors in determining whether larvae are likely to be exposed to *Bt* maize pollen. The importance of the temporal coincidence of the feeding period of sensitive larval stages with maize pollen shed for the determination of the individual risks for non-target butterfly species has been discussed also by Holst et al. (2013). In ERA practice, the conclusions on the temporal and spatial overlaps therefore have to consider not only the specific biology of the larvae but also the specific phenology of the maize variety which is also likely to vary across cultivation areas in Europe.

Conclusions

The analysis presented in this study is based on the actual distribution information of selected FFH butterfly species and data on maize cultivation in six different BGRs of Europe. It provides an assessment of the distribution of EU-wide protected non-target lepidopteran species in different BGRs and of the potential spatial overlap between these species and maize cultivation areas in the EU. The analysis shows that the potential spatial exposure of several protected butterfly species in Europe to *Bt* maize cultivation cannot be excluded at the BGR level.

The literature- and GIS-based selection approach for evaluating overlaps of maize cultivation and the occurrence of FFH lepidopteran species presented in this study is considered useful for the ERA of insect-resistant (*Bt*) maize. The approach can be used in the first step of the ERA, the problem formulation, in order to select those EU-wide protected species that may be most likely at risk when *Bt* maize is cultivated throughout the EU. It helps further structuring the ERA by indicating which species are likely to be exposed to the relevant GMO due to their occurrence and distribution in each BGR. However, further ERA steps require a more detailed analysis of actual spatial overlaps of protected lepidopteran species with maize cultivation areas with a finer spatial resolution. In addition, temporal overlaps of larval occurrences with regional maize flowering periods as well as species specificities have also to be taken into account. However, such an analysis is not feasible at an EU-wide scale and will require an involvement of national authorities and biodiversity experts.

The approach proposed in this study can be used as a tool for the consideration of different receiving environments based on the biogeographical regions during the ERA of GMOs which is transferable to other types of GMOs as well as other protected species or habitats.

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